PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P24533WO/MRM	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/003904	International filing date *(day/month/year)* 13.09.2004	Priority date *(day/month/year)* 11.09.2003
International Patent Classification (IPC) or national classification and IPC A61K47/48		
Applicant HEALTH PROTECTION AGENCY et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 14 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 8 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29.03.2005	18.01.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Dullaart, A Telephone No. +31 70 340-3290

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003904

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-49	as originally filed
Claims, Numbers	
1-46	received on 08.09.2005 with letter of 06.09.2005
Drawings, Sheets	
1/8-8/8	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-18 in part, and 19-46

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1-18 in part are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☒ the claims, or said claims Nos. 1-18 in part are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-18 in part, and 19-46

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☒ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1, 2 and part of 17 and 18 .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-18
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III.

In the present application, the International Searching Authority has restricted the search because of the following objections under Articles 5 and 6 PCT.

Independent claim 1, as well as most of the dependent claims, encompass a genus of compounds defined only by their function, wherein the relationship between the structural features of the members of the genus and said function has not been defined. In the absence of such a relationship either disclosed in the as-filed application or which would have been recognised based upon information readily available to one skilled in the art, the person skilled in the art would not know how to make and use compounds that lack structural definition. The fact that one could have assayed a compound of interest using the claimed assays does not overcome this defect since one would have no knowledge beforehand as to whether or not any given compound (other than those that might be particularly disclosed in an application) would fall within the scope of what is claimed. It would require undue experimentation (be an undue burden) to randomly screen undefined compounds for the claimed activity. Therefore, claims 1-18 do not fulfil the requirements of Articles 5 and 6 PCT.

As it is not possible to form an opinion on unsearched subject-matter, the following must be limited accordingly.

Re Item IV.

The separate inventions/groups of inventions are:

No.	Claims	Subject
1.	17-18 in part, 1 and 2	Method of designing a non-cytotoxic toxin as defined by these claims
2.	17-18 in part, and 3-16	Method of identifying an agonist as defined in these claims.
3.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of

		the agent is IL-13
4.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of the agent is insulin
5.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of the agent is mast cell degranulating peptide.
6.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of the agent is IL-4
7.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of the agent is TNF α
8.	19-32 and 37-46 in part	Pharmaceutical composition and its use (in a method of treatment) as defined in these claims, in which the TM of the agent is EGF.
9.	33-34	DNA construct as defined in claim 44, and its use in the preparation of a chimeric agent as defined in claim 45
10.	35-36	Process of preparing a conjugate as defined in these claims, i.e., by conjugation rather than by expression of a single DNA construct.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The problem underlying the present application is to provide new medicaments for therapy. As a solution, the applicant provides the reader with a method of designing a non-cytotoxic toxin conjugate as defined by originally filed claims 1-13, 28 and 29. As a result of this method, conjugates are designed, which can be used in the pharmaceutical compositions defined by originally filed claims 30-43. The actual preparation of these active agents is defined in claims originally filed 45-47. The use of the pharmaceutical composition is defined by claims originally filed 48-57.
In order to be able to perform the method of designing the conjugate, the applicant has provided the reader with a test to select one of the components of the conjugate in

originally filed claims 14-29. This component is used as the targeting moiety (TM).
The DNA construct defined in originally filed claim 44 is necessary for the methods of preparing according to originally filed claim 45.
The technical feature which a priori seems to link all these features is found in the ability of the binding domain to target the remaining components of the conjugate to different cells than the toxin as a whole would be targeted, or to test if a moiety, used for targeting, has the ability to stimulate secretion.

The ability of certain agents, known to stimulate secretion e.g. from present example 1, to target a toxin, has already been described in the prior art.
JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 270, no. 28, 14 July 1995 (1995-07-14), pages 16775-16780, XP002011861 ISSN: 0021-9258 describes the conjugate of IL-13 with a Pseudomonas Exotoxin mutant. As is clear from page 16775, right-hand column, the binding domain of the pseudomonas exotoxin has been removed and replaced by IL-13, like in the present application.
In **WO 98/07864 A**, the TM is IGF-1, attached to LH(N) (see the examples, especially example 3= LHN/A, and the figures).
In **WO 94/21300 A**, claim 14 defines a conjugate, in which TM is IGF-II, the protease (called: domain E) is "the domain or domain fragment of the Light chain of botulinum neurotoxin having Zn dependent metalloprotease activity", and the Translocation domain (domain T) is "the domain or domain fragment of the botulinum neurotoxin Heavy chain responsible for trans location of the toxin across the cell membrane"
These documents also describe different processes for preparing such agents. Therefore, the processes of preparation as defined by claims 45-47 must be seen as alternatives to these prior art processes, each defined by their specific possible contribution over the prior art.

Also, several compounds eligible as TM have been tested in the prior art for their ability to stimulate secretion.
INFLAMMATION RESEARCH, vol. 49, no. 4, April 2000 (2000-04), pages 162-169, XP008043079 ISSN: 1023-3830 describes, that mucin secretion triggered by IL-1, IL-6 or TNF-α.
The authors of **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE**

UNITED STATES OF AMERICA, vol. 98, no. 6, 13 March 2001 (2001-03-13), pages 3561-3566, XP008043078 ISSN: 0027-8424 show, that insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis.
AMERICAN JOURNAL OF PHYSIOLOGY - LUNG CELLULAR AND MOLECULAR PHYSIOLOGY, UNITED STATES, vol. 276, no. 4 20-4, 1999, pages L596-L603, XP008043082 ISSN: 1040-0605 tests the stimulation of the secretion of mucin by IL-4. Like in the present application, LS180 cells and NHTBE cells were used to determine this influence.
GASTROENTEROLOGY, vol. 114, no. 4 PART 2, 15 April 1998 (1998-04-15), page A973, XP008043029
& DIGESTIVE DISEASES WEEK AND THE 99TH ANNUAL MEETING OF THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION; NEW ORLEANS, LOUISIANA, USA; MAY 16-22, 1998 ISSN: 0016-5085 studies how three different cytokines affect the mucin secretion on LS180 cells. More specifically, the authors determine which mucin is secreted under the influence of these cytokines.
FASEB JOURNAL, vol. 11, no. 3, 1997, page A516, XP008043069
& ANNUAL MEETING OF THE PROFESSIONAL RESEARCH SCIENTISTS ON EXPERIMENTAL BIOLOGY 97; NEW ORLEANS, LOUISIANA, USA; APRIL 6-9, 1997 ISSN: 0892-6638 uses the cell line LS180 to determine that GTPγS stimulates mucin secretion.

In the light of these disclosures, both possible technical concepts, linking a priori the different subjects together, are known from the prior art.
Therefore, these technical features can no longer serve as special technical feature in the sense of Rule 13 PCT, linking the different subjects together.
Since there is no other technical feature, that could fulfil the role of special technical feature in the sense of Rule 13 PCT, the present application lacks unity of invention, containing the subject-matters as listed.
Although some document cited in the present search report may also be pertinent for further inventions mentioned this does not imply, that the search for those subjects has been fully performed.

In the search phase, the applicant has paid one single additional search fee. As a consequence, the search only covers inventions 1 and 2. Under chapter II, the applicant

did not pay an additional examination fee. Examination shall therefore be limited to the first invention mentioned in the claims.

In reaction to the Written Opinion of the International Searching Authority, the applicant has submitted amended claims stating, that the second invention is now claimed as dependent on the first solves the problem of unity of invention. However, if the applicant had wished to submit this argumentation as protest against the finding of lack of unity of invention, he should have paid an additional examination fee, which he did not.

In the claims as originally filed, the method of identifying the agonist as defined in the first invention was defined in general terms. In the second invention, only the method of detection was defined, without further implication in designing a non-cytotoxic toxin. For this reason, the objection for lack of unity between the two inventions is maintained. In principle, the International Preliminary Examination Authority is not obliged to perform an examination for the second subject as defined by the ISA. However, since the objections against the second are identical to those raised by the ISA, they are repeated under **Item V**.

In the listing of the different subjects above, the International Preliminary Examination Authority has indicated the claim numbers according to the newly submitted claims.

Re Item V.

1 Reference is made to the following documents:

D1: Enss M -L et al: "Proinflammatory cytokines trigger MUC gene expression and mucin release in the intestinal cancer cell line LS180" Inflammation Research, Vol. 49, no. 4, April 2000 (2000-04), pages 162-169, XP008043079 ISSN: 1023-3830

D2: Skeberdis Vytenis A et al: "Insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis" Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 6, 13 March 2001 (2001-03-13), pages 3561-3566, XP008043078 ISSN: 0027-8424

D3: Jayawickreme S P et al: "Regulation of 15-lipoxygenase expression and mucus secretion by IL-4 in human bronchial epithelial cells" American Journal of Physiology - Lung Cellular and Molecular Physiology, vol. 276, no. 4 20-4, 1999, pages L596-L603, XP008043082 ISSN: 1040-0605

D4: Enss M -L et al: "Proinflammatory cytokines differentially affect mucin expression in LS180 cells" Gastroenterology, Vol. 114, no. 4, part 2, 15 April 1998 (1998-04-15), page A973, XP008043029 & Digestive Diseases Week and the 99th Annual Meeting of the American Gastroenterological Association; New Orleans, Louisiana, USA; May 16-22, 1998, ISSN: 0016-5085

D5: Abdulla P et al: "GTP and Ca^{2+}-dependent mucin secretion in permeabilized LS180 human colonic cancer cells: Modulation by anion substitution" FASEB Journal, Vol. 11, no. 3, 1997, page A516, XP008043069 &; ANNUAL Meeting of the Professional Research Scientists on Experimental Biology 97; New Orleans, Louisiana, USA; April 6-9, 1997 ISSN: 0892-6638

D6: Debinski W et al: "A Novel Chimeric Protein Composed of Interleukin-13 and Pseudomonas Exotoxin is Highly Cytotoxic to Human Carcinoma Cells Expressing Receptors for Interleukin-13 and Interleukin-4" Journal of Biological Chemistry, American Society of Biological Chemists, Baltimore, MD, US, vol. 270, no. 28, 14 July 1995 (1995-07-14), pages 16775-16780, XP002011861 ISSN: 0021-9258

D7: WO 98/07864 A (MICROBIOLOGICAL RESEARCH AUTHORITY CAMR (CENTRE FO; THE SPEYWOOD LABOR) 26 February 1998 (1998-02-26)

D8: WO 94/21300 A (THE SPEYWOOD LABORATORY LTD; PUBLIC HEALTH LABORATORY SERVICE BOARD; N) 29 September 1994 (1994-09-29)

D9: EP 0 467 536 A (MERCK & CO. INC) 22 January 1992 (1992-01-22)

D10: Li Dailin et al: "Hyperosmolarity reduces GLUT4 endocytosis and increases its exocytosis from a VAMP2-independent pool in L6 muscle cells" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 25, 22 June 2001 (2001-06-22), pages 22883-22891, XP002329525 ISSN: 0021-9258

D11: Olson Ann Louise et al: "Insulin-mediated GLUT4 translocation is dependent on the microtubule network"

JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 14, 6 April 2001 (2001-04-06), pages 10706-10714, XP002329526 ISSN: 0021-9258

D12: Yang Chun Zhi et al: "ADP-ribosylation factor 6 (ARF6) defines two insulin-regulated secretory pathways in adipocytes"
JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 36, 3 September 1999 (1999-09-03), pages 25297-25300, XP002329527 ISSN: 0021-9258

D13: Cain C C et al: "Members of the VAMP family of synaptic vesicle proteins are components of glucose transporter-containing vesicles from rat adipocytes"
JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 17, 1992, pages 11681-11684, XP002329528 ISSN: 0021-9258

D14: Davis R J et al: "Insulin-like Growth Factor I and Epidermal Growth Factor regulate the expression of transferrin receptors at the cell surface by distinct mechanisms"
JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 262, no. 27, 1987, pages 13126-13134, XP002329529 ISSN: 0021-9258

2 Invention 1

Document **D1** discloses mucin secretion triggered by IL-1, IL-6 or TNF-α.
Document **D2** discloses that insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis.
Document **D3** discloses the stimulation of the secretion of mucin by IL-4. Like in the present application, LS180 cells and NHTBE cells were used to determine this influence.
Document **D4** discloses how three different cytokines affect the mucin secretion on LS180 cells. More specifically, the authors determine which mucin is secreted under the influence of these cytokines.
Document **D5** discloses the use of the cell line LS180 to determine that GTP-gamma-S stimulates mucin secretion.
Document **D6** discloses the conjugate of IL-13 with a Pseudomonas Exotoxin mutant. As is clear from page 16775, right-hand column, the binding domain of the pseudomonas exotoxin has been removed and replaced by IL-13, like in the present application.
Document **D7** discloses conjugates, in which the targeting moiety (TM) is IGF-1, attached to LH(N) (see the examples, especially example 3= LHN/A, and the figures).

In document **D8**, claim 14 defines a conjugate, in which TM is IGF-II, the protease (called: domain E) is "the domain or domain fragment of the Light chain of botulinum neurotoxin having Zn dependent metalloprotease activity", and the Translocation domain (domain T) is "the domain or domain fragment of the botulinum neurotoxin Heavy chain responsible for translocation of the toxin across the cell membrane"
Document **D9** discloses in the examples one of the possible results of the methods as defined by the first invention.

The method claimed in the first invention has not been described in these documents, and therefore meets the requirements of Article 33.3 PCT for novelty.

The presently claimed method can be distinguished from the methods according to any of **D6** to **D9** by the fact, that the TM is first tested for its ability to stimulate secretion. This test, however, is known to the skilled person from each of **D1** to **D5**. Moreover, the applicant has not shown any effect of previously testing e.g. IL-13 for its ability to stimulate mucin secretion in LS180 cells (as in example 1 of the present application) on the designing procedure. Therefore, as no technical effect can be ascribed to this previously performed test, the selection procedure is a mere alternative to the one performed in **D6**, void of an inventive step in the sense of Article 33.3 PCT.

3 Invention 2

Document **D1** discloses mucin secretion triggered by IL-1, IL-6 or TNF-alpha.
Document **D2** discloses that insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis.
Document **D3** discloses the stimulation of the secretion of mucin by IL-4. Like in the present application, LS180 cells and NHTBE cells were used to determine this influence.
Document **D4** discloses how three different cytokines affect the mucin secretion on LS180 cells. More specifically, the authors determine which mucin is secreted under the influence of these cytokines.
Document **D5** discloses the use of the cell line LS180 to determine that GTP-gamma-S stimulates mucin secretion.
Document **D10** discloses that exocytosis of the glucose transporter 4 (GluT4) is stimulated by insulin.

Document **D11** discloses that exocytosis of the glucose transporter 4 (GluT4) is stimulated by insulin.
Document **D12** discloses the role of ARF6 in the exocytosis of the glucose transporter 4 (GluT4), stimulated by insulin.
Document **D13** discloses by which mechanism exocytosis of the glucose transporter 4 (GluT4) is stimulated by insulin.
Document **D14** discloses that exocytosis of the transferrin receptors is stimulated by EGF.

These documents all describe tests by which the skilled person can verify if a compound of interest does indeed stimulate the exocytic function of a cell. In **D1** to **D5**, the same LS180 cells are used as in the present application. Moreover, the tests thus known from the prior art mention the exocytic activity of specifically claimed TM of the present application. In certain cases, like for insulin in **D12** or **D13**, the underlying mechanism is further detailed. In view of this prior art, the second invention as claimed in the present application does not fulfil the requirements of Article 33.2 PCT for novelty.

Re Item VIII.

Independent claim 1, as well as most of the dependent claims, encompass a genus of compounds defined only by their function, wherein the relationship between the structural features of the members of the genus and said function has not been defined. In the absence of such a relationship either disclosed in the as-filed application or which would have been recognised based upon information readily available to one skilled in the art, the person skilled in the art would not know how to make and use compounds that lack structural definition. The fact that one could have assayed a compound of interest using the claimed assays does not overcome this defect since one would have no knowledge beforehand as to whether or not any given compound (other than those that might be particularly disclosed in an application) would fall within the scope of what is claimed. It would require undue experimentation (be an undue burden) to randomly screen undefined compounds for the claimed activity.
It is to be noted that this objection not only concerns the definition of the TM, but also the definition of both other moieties (i.e., the translocation domain and the protease).
Therefore, claims 1-18 do not fulfil the requirements of Articles 5 and 6 PCT.

Claims

1. A method of designing a non-cytotoxic toxin conjugate for inhibition or reduction of exocytic fusion in a target cell, which method comprises: -

(A) identifying an agonist that increases exocytic fusion in said target cell; and

(B) preparing an agent, which agent includes:-

(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on said target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist identifiable by step (A);

(ii) a non-cytotoxic protease or a fragment thereof, which protease or protease fragment is capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

(iii) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

2. A method of designing a non-cytotoxic toxin conjugate for inhibition or reduction of exocytic fusion in a target cell, which method comprises:-

(A) identifying an agonist that increases exocytic fusion in said target cell; and

(B) preparing an agent, which agent includes:-

(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on said target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist identifiable by step (A);

(ii) a DNA sequence encoding a non-cytotoxic protease or a fragment thereof, which DNA sequence is expressible in the target cell and when so expressed provides a protease or protease fragment capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

(iii) a Translocation Domain that translocates the DNA sequence encoding the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

3. A method according to Claim 1 or Claim 2, wherein said step of identifying an agonist comprises identifying an agonist that is suitable for re-targeting the non-cytotoxic protease or a fragment thereof to a target cell, said method comprising:-

(A) identifying a putative agonist molecule;

(B) contacting the target cell with said putative agonist molecule; and

(C) confirming that said putative agonist molecule is an agonist by identifying an increase in exocytic fusion in the target cell when said molecule is present compared with when said molecule is absent.

4. A method according to Claim 3, comprising the step of confirming that the putative agonist molecule or agonist is capable of being combined with a non-cytotoxic protease (or a fragment thereof) or a DNA sequence encoding said protease (or the fragment thereof) to form an agent of the present invention.

5. A method according to Claim 3 or 4, comprising the step of confirming that said putative agonist molecule or agonist binds to a Binding Site on the target cell, which Binding Site is susceptible to receptor-mediated endocytosis.

6. A method according to any of Claims 3-5, comprising the step of confirming that said putative agonist molecule or agonist is able to deliver said non-cytotoxic protease (or fragment thereof), or a DNA sequence encoding said protease (or the fragment thereof), into the cytosol of a target cell.

7. A method according to any of Claims 3-6, wherein step (C) comprises detecting an increase in secretion from the target cell when agonist is present compared with when said agonist is absent.

8. A method according to Claim 7, wherein said detecting is performed by an assay employing chromatography, mass spectroscopy, and/or fluorescence.

9. A method according to Claim 7 or 8, wherein said detecting is performed by an assay employing ELISA/EIA/RIA techniques, and/or radio-tracer techniques.

10. A method according to any of Claims 3-6, wherein step (C) comprises detecting an increase in the concentration of a cell membrane protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

11. A method according to Claim 10, wherein the cell membrane protein is a cell receptor protein, and the method comprises detecting an increase in the concentration of said receptor protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

12. A method according to Claim 10 or 11, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, fluorescent-ligand binding techniques, and/or radio-ligand binding techniques.

13. A method according to Claim 10, wherein the cell membrane protein is a transporter protein, and the method comprises detecting an increase in the concentration of said transporter protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

14. A method according to Claim 10 or 13, wherein said detecting is performed by an assay employing immuno-histochemistry, flow cytometry, western blotting of isolated plasma membrane cell fractions, and/or intra- and extracellular assessment of transported material (eg. glucose).

15. A method according to Claim 10, wherein the cell membrane protein is a membrane channel protein, and the method comprises detecting an increase in the concentration of said membrane channel protein expressed at the surface of the target cell when agonist is present compared with when said agonist is absent.

16. A method according to Claim 10 or 15, wherein said detecting is performed by an assay employing biochemical assessment of ion concentration in an isolated sample (eg. serum, plasma, or urine), electrophysiology of tissue (eg. *ex vivo* tissue), intra- and extracellular assessment of transported material (eg. glucose),

immuno-histochemistry, flow cytometry, and western blotting of isolated plasma membrane cell fractions.

17. A method according to any preceding claim, wherein the protease is a bacterial protein, or a fragment thereof capable of cleaving a protein of the exocytic fusion apparatus of the target cell.

18. A method according to Claim 17, wherein the bacterial protein is selected from a clostridial neurotoxin, or an IgA protease.

19. A pharmaceutical composition, which includes an agent comprising:-

(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on a target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist that is capable of increasing exocytic fusion in the target cell;

(ii) a non-cytotoxic protease or a fragment thereof, which protease or protease fragment is capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

(iv) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

20. A pharmaceutical composition, which includes an agent comprising: -

(i) a Targeting Moiety (TM) that binds the agent to a Binding Site on a target cell, which Binding Site undergoes endocytosis to be incorporated into an endosome within the target cell, and wherein the TM is an agonist that is capable of increasing exocytic fusion in the target cell;

(ii) a DNA sequence encoding a non-cytotoxic protease or a fragment thereof, which DNA sequence is expressible in the target cell and when so expressed provides a protease or protease fragment capable of cleaving a protein of the exocytic fusion apparatus of said target cell; and

(iii) a Translocation Domain that translocates the protease or protease fragment from within the endosome, across the endosomal membrane, and into the cytosol of the target cell.

21. A composition according to Claim 19 or 20, wherein the agonist is capable of contacting the target cell and increasing secretion from said target cell compared with when the agonist is absent.

22. A composition according to Claim 19 or 20, wherein the agonist is capable of contacting the target cell and increasing the concentration of a cell membrane protein expressed at the cell surface of said target cell compared with when the agonist is absent.

23. A composition according to Claim 22, wherein the agonist is capable of contacting the target cell and increasing the concentration of a cell receptor protein expressed at the cell surface of said target cell compared with when the agonist is absent.

24. A composition according to Claim 22, wherein the agonist is capable of contacting the target cell and increasing the concentration of a transporter protein expressed at the surface of said target cell compared with when the agonist is absent.

25. A composition according to Claim 22, wherein the agonist is capable of contacting the target cell and increasing the concentration of a membrane channel protein expressed at the surface of said target cell compared with when the agonist is absent.

26. A composition according to any of Claims 19-25, wherein said agent has been prepared by a method according to any of Claims 1-13.

27. A composition according to any of Claims 19-25, wherein said agonist has been identified by a method according to any of Claims 3-16.

28. A composition according to any of Claims 19-27, further comprising an inhibitor that alleviates, in a patient, clinical symptoms caused by exocytic fusion in said target cell.

29. A composition according to Claim 28, wherein the inhibitor alleviates the clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.

30. A composition according to Claim 28 or 29, wherein the inhibitor has a short-acting duration once administered to a patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.

31. A composition according to any of Claims 19-30, wherein the protease is a bacterial protein, or a fragment thereof capable of cleaving a protein of the exocytic fusion apparatus of the target cell.

32. A composition according to Claim 31, wherein the bacterial protein is selected from a clostridial neurotoxin, or an IgA protease.

33. A DNA construct encoding the agent defined in Claim 19, said construct comprising a DNA encoding the TM and/or the Translocation Domain, and the protease (or fragment thereof).

34. A method of preparing the agent defined in Claim 19, comprising expressing the DNA construct of Claim 33 in a host cell.

35. A method of preparing the agent defined in Claim 19, comprising covalently linking the TM and/or Translocation Domain, and the protease (or fragment thereof).

36. A method of preparing the agent defined in Claim 20, comprising covalently linking the TM and/or Translocation Domain, and the DNA sequence encoding the protease (or the fragment thereof).

37. Use of a composition according to any of Claims 19-32 for the manufacture of a medicament for treating a medical disease or condition in a patient, wherein the disease or condition is caused by exocytic fusion in a target cell of said patient

38. Use of a composition according to any of Claims 19-27 for the manufacture of a medicament for treating a medical disease or condition in a patient, wherein the disease or condition is caused by exocytic fusion in a target cell of said patient.

39. Use of a composition according to Claim 38, wherein the medicament is to be administered to the patient prior to, simultaneously with, or subsequent to an inhibitor, and wherein the inhibitor alleviates, in the patient, clinical symptoms caused by exocytic fusion.

40. Use of a composition according to Claim 39, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.

41. Use of a composition according to Claim 39 or 40, wherein the inhibitor has a short-acting duration once administered to the patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.

42. A method for treating a medical disease or condition caused by exocytic fusion in a target cell, comprising administering to a patient a composition according to any of Claims 19-32.

43. A method for treating a medical disease or condition caused by exocytic fusion in a target cell, comprising administering to a patient a composition according to any of Claims 19-27.

44. A method according to Claim 43, wherein the composition is administered to a patient prior to, simultaneously with, or subsequent to an inhibitor, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by exocytic fusion.

45. A method according to Claim 44, wherein the inhibitor alleviates, in the patient, clinical symptoms caused by increased exocytic fusion resulting from binding of the agonist to the target cell.

46. A method according to Claim 44 or 45, wherein the inhibitor has a short-acting duration once administered to the patient, preferably a short-acting duration of 1-3 days, more preferably a short-acting duration of 1-2 days, most preferably a short-acting duration of 24-36 hours.